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EXPERIMENTAL STUDIES ON THE DURATION OF LIFE

III. THE EFFECT OF SUCCESSIVE ETHERIZATIONS ON THE DURATION OF LIFE OF DROSOPHILA[1]

PROFESSOR RAYMOND PEARL AND SYLVIA L. PARKER

PURPOSE AND PLAN OF EXPERIMENTS

IN any experimental work of a genetic character on *Drosophila,* it is often necessary to anesthetize the flies which are to be used in an experiment for a sufficiently long time so that they may be sexed and sorted into different groups for the purpose of making matings, etc. It has been shown by Morgan (33) that this procedure has no effect upon the causation of morphological mutations, the inheritance of which he has studied (9). The effect might, however, conceivably be quite different in the case of a physiological character like duration of life. Any one who has undergone a major surgical operation feels that anesthetization is at least immediately a rather profound physiological disturbance. Unfortunately, so far as we are aware, no accurate determinations have ever been made to show whether in man one or more anesthetizations changes the expectation of life. As a matter of fact, there are presumably no human data on the point available in any such amount as would be necessary for actuarial determinations, because in man anesthetization is, generally speaking, only undertaken in connection with surgical operations of greater or less severity, so that if we did have statistics of expectation of life of persons who had been anesthetized, there would always be involved the two factors of anesthetization and operation. In *Drosophila* these two factors can be separated.

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[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 54. For description of the method of numbering bibliographic citations see the second paper in the series (32).

It has seemed important, in an early stage of our experimental work on the duration of life in this form, to make a careful and extensive experimental test of the question of whether anesthetization singly or repeated changed in any way the expectation of life or form of the life curve, so that if this factor does have any significant influence, either favorable or unfavorable, due allowance may be made for it. It is the purpose of this paper to report the results of such a test.

The flies used in this experiment were flies of line 107 (generation 8 since January 14, 1921, line bred from a single brother and sister mating for approximately 30 generations). The characteristics of this line relative to duration of life have already been described (*cf.* Pearl and Parker (32)). The 4,330 flies used emerged between 10 A.M. April 18, 1921, and 4 P.M. April 22, 1921, from thirty-five mass cultures started in half-pint milk bottles April 7, 1921. The regular procedure in these experiments was to collect the flies from all 35 breeding bottles in one empty bottle and then to count the flies through a counting tube into 1-ounce vials, allowing 50 flies to each vial.[2] Ten vials were used for each series, except the control series, which had 18. For two of the series only two hours were allowed between successive emptyings of the mating bottles, to get flies at an average age of one hour, assuming that they emerged uniformly over the interval. One series was etherized as soon as counted out, and the other series kept as a special control group to see if the handling when the flies were so young and soft had any effect on the duration of life. For the rest of the series the flies were allowed to emerge over a 24-hour interval. Each day's hatch was divided randomly and as equally as possible among the different series.

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[2] It will be noted that the totals shown in Table I do not accord exactly with this statement. The discrepancies are due to the fact that a few flies were lost in changing to fresh bottles in the course of the life duration determinations made according to the technique described in (27), and occasionally a bottle was broken by accident and all its contained flies lost.

The counting tube referred to above is a device invented in this laboratory which we find extremely useful in a great deal of the experimental work. It was devised and first used in connection with studies of the growth of experimental populations of *Drosophila* (*cf.* Pearl (7), and Pearl and Kelly (34)). Its construction is shown in Fig. 1.

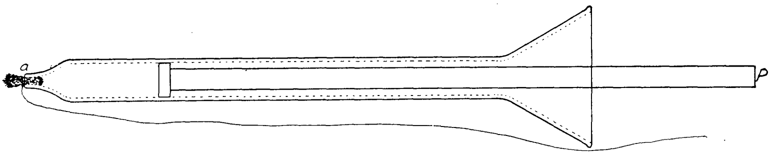

FIG. 1. Diagram showing construction of *Drosophila* counting tube. The aperture at *a* is just large enough to allow one fly to pass through at a time. The essential dimensions are as follows: length over all 25 cm., diameter of main tube 2 cm., diameter of funnel mouth 6 cm.

When it is desired to count a definite number of flies the small aperture *a* is temporarily plugged with a bit of cotton wool, the plunger *P* is removed from the tube and flies are shaken into the counting tube by inverting the open bottle containing them over the funnel mouth of the counting tube. Then the plunger is inserted and gently moved forward to concentrate the flies in the lower end of the counting tube. Then the counting tube with enough cotton around it to close up the mouth of the bottle is inserted into the bottle into which it is desired to place the counted flies and the plug removed from the aperture *a*. Then as the flies come out of the tube, one by one, through the aperture *a*, they are counted as they pass this point, with the aid of a tally register, such as is used by doorkeepers at theaters, etc. The plunger is gently moved forward as necessary to keep up an even flow of flies through the mouth of the tube.

The ether dose used was constant for all the flies throughout the experiment. The group to be etherized was shaken into a clean half pint milk bottle; 5 c.c. of ether was poured onto a piece of absorbent cotton fastened to the under side of a cork stopper; the bottle with the flies was stoppered tightly with the cork and left for two minutes. Then the flies were turned out on a tile and sexed and counted (since that operation corresponds in extent of handling to what we need to do in making up matings, etc.), then emptied into a vial with fresh food, where they recovered from the ether in about half an hour. For each successive group of flies a fresh bottle and fresh cotton for the ether were of course used.

In all other, here unspecified, particulars the technique used in these ether experiments was uniformly that described in detail in the first paper of this series (27).

Seven series of experiments were conducted, differing in respect of the number of times the flies were etherized, and in their age at the time of etherization. The seven series were as follows:

A. Etherized once when one hour of age.
B. Etherized once when twelve hours of age.
C. Etherized once when thirty-six hours of age.
D. Etherized once when three and a half days of age.
E. Etherized twice when seven, and fourteen days of age, respectively.
F. Etherized three times when seven, fourteen, and twenty-one days of age, respectively.
G. Etherized four times when seven, fourteen, twenty-one and twenty-eight days of age, respectively.

DATA

The l_x lines for the several series of etherized flies and the controls are given in Table I. These l_x distributions are calculated on the basis of 1,000 flies at emergence from the pupal stage, with the absolute number of flies on which the distribution is based given at the bottom of the column in each case.

The l_x distributions for all etherized flies and for their controls in the ether experiment, and for two tests of the flies in line 101 and its continuation 107, are shown graphically in Fig. 2. The data for the survivorship lines in the two tests of line 107 are to be found in Pearl and Parker (27).

TABLE I

SURVIVAL DISTRIBUTION OF ETHERIZED AND NON-ETHERIZED DROSOPHILA CULTURES

Age in Days	Etherized Series								All Controls	Controls 1 hr. Old	Controls 12hrs Old
	A	*B*	*C*	*D*	*E*	*F*	*G*	All Etherized			
1- 6	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
7-12	988	998	988	998	993	990	995	993	985	990	983
13-18	986	993	978	995	984	983	995	988	980	987	976
19-24	984	971	978	984	971	971	964	975	974	981	970
25-30	970	953	966	965	957	918	910	949	925	918	928
31-36	942	916	912	942	944	910	857	918	903	895	907
37-42	902	880	856	926	901	879	810	880	854	843	859
43-48	778	722	796	847	793	763	698	771	735	753	724
49-54	629	634	732	725	643	598	605	652	604	701	550
55-60	453	492	625	581	465	462	476	507	454	554	398
61-66	117	95	397	301	153	249	145	183	150	138	157
67-72	47	34	136	116	79	165	71	92	53	71	43
73-78	12	9	29	37	22	65	17	27	15	19	13
79-84	0	0	0	0	0	0	0	0	0	0	0
Absolute number of flies....	428	443	411	432	445	413	420	2,992	1,338	478	860

It is at once evident, from an examination of the figures in Table I, and the diagram, that there was no considerable difference in duration of life, or in the form of the life curve, for the etherized flies taken as a class, and the non-etherized groups. It is, however, desirable to examine the results of the experiments in detail in order to see whether there are detectable by biometric methods any small but still statistically significant differences between the several groups. To this end, Table II has been prepared, giving the usual biometric constants for the several series.

Comparing first the entire etherized group as a whole with those which never had any ether at all in their lives, it is seen that the mean duration of life (expectation of life at emergence from pupa) is 1.82 ± .30 days longer in the former (etherized) than in the latter (normal) group. The difference is thus slightly more than 6 times

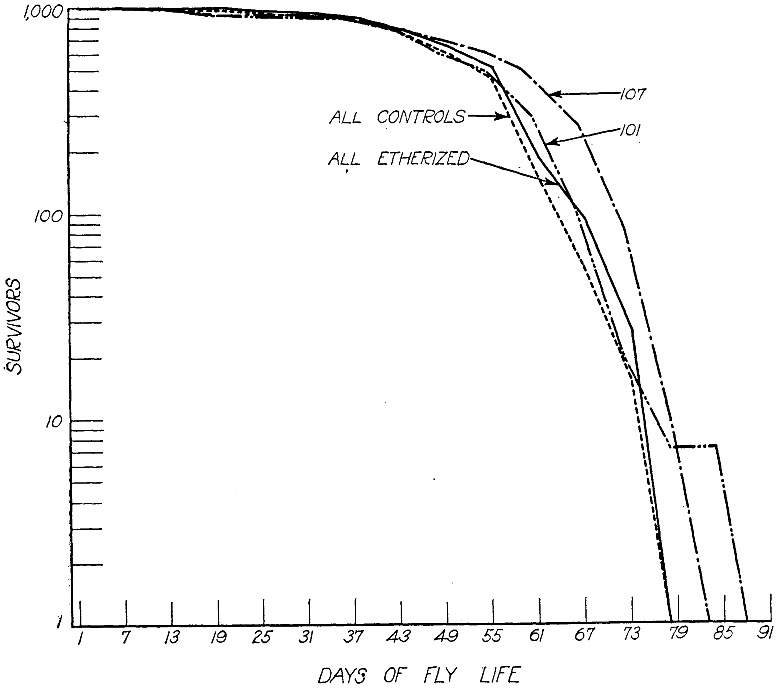

FIG. 2. The l_x lines for all etherized and controlled flies, plotted from the data of Table I.

TABLE II

BIOMETRIC CONSTANTS FOR DURATION OF LIFE OF ETHERIZED AND NORMAL DROSOPHILA

Treatment	Number of Flies	Mean (in days)	Standard Deviation (in days)	Coefficient of Variation
All etherized	2,992	51.60 ± .16	13.30 ± .12	25.77 ± .24
All controls	1,338	49.78 ± .25	13.68 ± .18	27.47 ± .38
Etherized when 1 hour old	428	50.82 ± .38	11.76 ± .27	23.14 ± .56
Etherized when 12 hours old	443	50.20 ± .39	12.25 ± .28	24.41 ± .59
Etherized when 36 hours old	411	53.36 ± .46	13.91 ± .33	26.06 ± .65
Etherized when 3½ days old	432	54.50 ± .40	12.36 ± .28	22.68 ± .55
Etherized when 7 and 14 days old	445	51.43 ± .40	12.50 ± .28	24.31 ± .58
Etherized when 7, 14, and 21 days old	413	51.72 ± .50	15.01 ± .35	29.03 ± .74
Etherized when 7, 14, 21, and 28 days old	420	49.26 ± .47	14.42 ± .34	29.26 ± .74
Controls (taken out of mating bottles when 1 hour old)	478	51.11 ± .42	13.75 ± .30	26.90 ± .63
Controls (taken out of mating bottles when 12 hours old)	860	49.04 ± .31	13.58 ± .22	27.69 ± .48

its probable error, and must therefore be regarded as statistically significant. Absolutely, however, the difference is small. It is equivalent to only 3.7 per cent. increase of the expectation of life of the controls. In variability in respect of duration of life there is plainly no significant difference between etherized and control groups.

It is not entirely clear that the small difference between the etherized and control groups in mean duration of life can be regarded as due to the influence of the ether. An examination of the last two lines of Table II shows that an entirely similar difference in the means appears between the two control groups, which differ only in respect of the time when they were taken from the mating bottles, and without either having been etherized. The difference in these two means amounts to 2.07 ± .52 days, a statistically significant and absolutely slightly larger difference than that between etherized and control groups. Again there is no significant difference in variability in the two groups.

Altogether we shall be justified in concluding that there is no evidence from these experiments that the occasional etherization of *Drosophila* to the extent necessary in sexing and making matings alters the expectation of life by an amount large enough to introduce any sensible source of error into experiments on the duration of life in this form, except possibly where the most careful and accurate actuarial determinations need to be made. Then it will be well to have this possible source of error in mind and to plan the experiments in such way as to check it.

Examining the results for the different etherized series it is seen that the highest mean duration of life appears in the group etherized once at 3½ days of age, and next to this stands the group etherized once at 1½ days of age. Both of these give relatively high mean values. There also appears a definite, though not particularly marked tendency for the variability in duration of life to be greater in the groups which were etherized several times. No great importance is probably to be attached to these differences between the several groups, however, though some of them appear significant statistically.

CONCLUSION

From the experiments herein described, involving the determination of the total duration of life in 4,330 individual flies, it may be safely concluded that no sensible error will be introduced into duration of life experiments on *Drosophila* as a result of completely anesthetizing the flies with ether, at least up to as many as four times in the course of their lives.

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